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Date: December 30, 2009 Rebecca A. Bellas

Rebecca A. Bellas

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application

Applicant:	Kusama	:	Art Unit:	1637
Serial No.:	10/826,119	:	Examiner:	Suryaprabha Chunduru
Filed:	April 16, 2004	:		

Title: OLIGONUCLEOTIDE SEQUENCES THAT IDENTIFY SPECIES OF ANIMAL

DECLARATION UNDER 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

I, Dr. Koichi Kadowaki, declare and say as follows:

I am an inventor of the claims of the above-identified patent application. I hold a Ph.D. degree in plant molecular biology awarded in 1989 from Kyoto University. I have conducted molecular biology research continuously since 1984 at the following professional positions:

1984 National Institute of Agrobiological Resources (Japan)/Researcher

1990 North Carolina State University/Post Doctoral Fellow
1991 National Institute of Agrobiological Resources (Japan)/Researcher
1997 Agency of Science and Technology, Section of Life Science (Japan)
1998 National Institute of Agrobiological Sciences (Japan)/Head of Laboratory
2006 Headquarters (Secretary of Research Council) Ministry of Agriculture, Forestry and Fisheries (Japan)/General Officer
2009 National Institute of Agrobiological Sciences (Japan)/Research Director-General

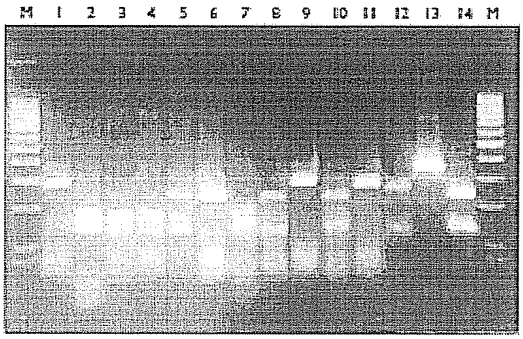
Before the oligonucleotide primers and methods disclosed in the above-referenced patent application, the only known method for determining the species origin of a target DNA in a food sample relied upon PCR followed by creation of a restriction map by RFLP (restriction fragment length polymorphism). RFLP techniques involve observing many bands formed on an electrophoretic gel created by endonuclease restriction of a PCR product. Therefore, the technician performing the determination must identify the restriction digest DNA bands among many such bands by comparison with a molecular weight ladder.

Fig. 1, below, shows exemplary results for PCR followed by RFLP using the BIOFOOD Identification Kit (Funakoshi (Japan)). The BIOFOOD Identification Kit was the most common kit for detecting animal species-derived DNA in 1999 (the year of the Lowe et al publication); however, sales of the BIOFOOD Identification Kit have now stopped.

The BIOFOOD Identification Kit is for the identification of animal species for animal derived components in a sample. First, the primer pair against a sequence common to vertebrates is used to perform PCR, and then the amplified DNA is digested with three restriction enzymes and subjected to gel electrophoresis. Animal species for animal-derived components in the sample are identified based on the electrophoretic patterns of the RFLP. That is, the PCR product formed using the primer pair against a sequence common to vertebrates in PCR is similar in all species; restriction digest is needed to identify the source of the PCR product. Sensitivity is form about 0.5 to 5% or

more of the sample. For a sample containing components derived from three or more animal species, it may be difficult to interpret the electrophoretic pattern.

Fig. 1: Exemplary Identification of Animal Species using PCR followed by RFLP.



Lane 1: buffalo; Lane 2: rabbit; Lane 3: horse; Lane 4: roe deer; Lane 5: cattle; Lane 6: Goat; Lane 7: deer; Lane 8: emu; Lane 9: red kangaroo; Lane 10: ostrich; Lane 11: cat; lane 12: dog; lane 13: muse; lane 14: human; lane M: 100 bp size marker. Source: BIOFOOD Identification Kit product information sheet.

As can be observed in Fig. 1, the result for each lane is very complex, which is compounded if a sample contains DNA from multiple species. On the contrary, the oligonucleotide primers and methods disclosed in the above-referenced patent application produce only a single band by PCR that is either present or not present depending upon the existence of a target DNA from a specific species or group of species (i.e., ruminants) in a sample. Thus, interpretation of the results is greatly simplified.

A product information sheet for the BIOFOOD Identification Kit from Funakoshi in Janpanese is attached as Appendix 1 along with a partial English translation. The partial English Translation includes a translation of the legend of Fig. 1.

As shown in Figs. 6 and 7 in the Specification of the above-referenced patent application, only a single band can be detected in a single lane of an electrophoretic gel, therefore, we can clearly and easily recognize the species origin by one view of the gel without specific comparison to a DNA weight ladder. This is an unexpected utility of the

primers described in the Specification. In addition, the claimed primer pairs can detect the target DNA as the same size band regardless of animal species, which serves as additional evidence that a positive result is not due to error. At present, the detection method using the claimed primer pairs is an official method authorized by Ministry of Agriculture, Forestry and Fisheries of Japan, listed in "The Official Methods of Feed Analysis", and widely utilized for detecting trace amounts of ruminant-derived DNA samples. The users of the claimed primer pairs are private enterprises, governmental agencies, and academic researchers.

Furthermore, extensive and laborious experimentation was required to obtain the claimed primer pairs. Appendixes 2 and 3 illustrate that the claimed primer pairs were obtained from extensive experimentation. As shown in Appendix 2, we have examined various combinations of primers for PCR for specific detection of ruminant-derived DNA. In each nucleotide sequence shown as "rumicon" or cases 1 to 8 in Appendix 2, a pair of boxes shows the regions of the ATPase8 gene where a primer pair binds in PCR in each experiment correspondingly labelled "rumicon" or cases 1 to 8 in Appendix 3. The "rumicon" is the claimed primer pair, Fpr-F and Fpr-R (SEQ ID NOS. 5 and 6). The cases 1 to 8 are the primer pairs which bind to regions near the region where the claimed primer pair binds. Using the primer pairs which were designed based on the boxed regions in Appendix 2, PCR was performed on various animal-derived DNA samples according to the same procedure as Example 3 in the Specification of the above-referenced patent application. The results are shown in Appendix 3.

In cases 1 to 7, some of the non-ruminant-derived DNAs were detected and some ruminant-derived DNAs could not be detected. In case 8, although sheep-derived DNA was detected, cattle-, goat-, or deer-derived DNA was not detected. In addition, in case 8, DNA derived from swine, which is not a ruminant, showed a positive band. In case 2, goat and deer (ruminants) are detected along with non-ruminants pig and horse, all with PCR products of approximately the same length. In case 1, ruminant-derived DNA from sheep and goat were not detected, and in case 6, ruminant-derived DNA from cattle (cow) and sheep were not detected. In case 2, which successfully detected sheep- and

goat-derived DNA, ruminant-derived DNA from cattle was not detected and ruminant-derived DNA from deer produced numerous bands rather than a single band. On the contrary, as show in the "rumicon" example of Appendix 3, which is the same as Fig. 7 of the Specification of the above-referenced patent application, the ruminant-derived DNAs could be detected and non-ruminant-derived DNAs were not detected with 100% accuracy. Thus, it is clear that the combination of primers employed significantly affects ruminant detection accuracy, where the claimed primer pair can specifically detect ruminant-derived DNA without false positive or negative results.

Therefore, it would not have been obvious to those skilled in the art to expect the claimed primers to have the unexpected property of accurate ruminant-derived DNA detection. That is, one skilled in the art could not predict that any of the primer sequences which are designed based on the boxed areas in Appendix 2 would have the functionality for accurate ruminant-derived DNA detection.

I, Koichi Kadowaki, hereby declare that all the statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued therein.

K. Kadowaki

Koichi Kadowaki, Ph.D.

Dec. 18, 2009.

Date

試料に混在する成分の動物種を同定できます
BIOFOOD Kit

未加工および加工済みの食品や飼料などの試料に存在する動物由来成分を検出し，動物種を同定するキットです。
試料から精製した DNA を，PCR で増幅後，アガロースゲル電気泳動で検出します。
※キットに DNA 精製用試薬は含まれません。

試料の破砕には「ミニコードレスグラインダー」や遠心分離によるホモジナイザー「バイオマッシャーII」が便利です。

Appendix 1

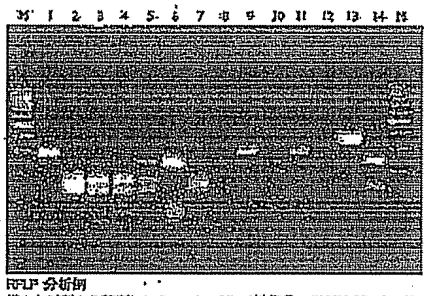
特 長

- BIOFOOD Identification Kit では，食品表示以外の肉類の混入の有無を検出し，またその動物種を同定することができます。
- タンパク質の検出を行うキットと異なり，試料中の DNA を検出するため，煮沸，化学的・物理的処理，粉砕，乾燥，加熱，本格などの加工をした後の試料からも検出できます。
- キットには，PCR による増幅に必要な試薬およびコントロール用 DNA が含まれており，PCR の偽陽性や，反応系への PCR 産物の混入を確認できます。

BIOFOOD Standard Kit
脊椎動物(哺乳類，鳥類，は虫類，両生類，魚類)に共通な配列に対するプライマーを用いた PCR を行い，これらの動物由来成分の存在の有無を確認するキットです。スクリーニングに適しています。
感度:0.5 % 以上

BIOFOOD Identification Kit
試料中の動物由来成分の動物種を同定するキットです。脊椎動物に共通な配列に対するプライマーを用いて PCR を行った後，3 種類の制限酵素で切断し，RFLP(Restriction Fragment LengthPolymorphism:制限酵素断片長多型)の電気泳動パターンにより動物種を同定します。
感度:0.5 ～ 5 % 以上
※3 種以上の動物由来成分が混在した試料の検査では，結果の判断が困難な場合があります。

BIOFOOD Mixed Kit
6 種類の動物(ウマ，ヒツジ，ウシ，ブタ，ニワトリ，ヤギ)それぞれに特異的なプライマーの混合物を用いた PCR により，これらの動物由来成分の有無を確認するキットです。上記のキットなどで既に検査を行った試料に対して，簡便に再検査できます。
感度:2 % 以上
※複数の動物由来成分が混在した試料の検査には適しておりません。



RFLP 分析例
様々な試料から精製した DNA を PCR で増幅後，BIOFOOD Identification Kit の Enzyme 1 で切断し，アガロースゲル電気泳動を行った。キットに含まれる別の制限酵素で処理した場合の結果と組み合わせることにより，動物種を特定できる。

lane 1 : スイギュウ
lane 2 : ウサギ
lane 3 : ウマ
lane 4 : シカ
lane 5 : ウシ
lane 6 : ヤギ
lane 7 : シカ
lane 8 : エミュー
lane 9 : アカカンガルー
lane 10 : ダチョウ
lane 11 : ネコ
lane 12 : イヌ
lane 13 : マウス
lane 14 : ヒト
lane M : 100 bp サイズマーカー

キット内容

共通

- $MgCl_2$ solution

- DNA polymerase

Standard Kit

- Master mix
- Mixed control material

Identification Kit

- Control material
- Master mix
- Restriction enzyme 1, 2, 3
- Enzyme buffer 1, 2, 3

Mixed Kit

- Multiplex master mix
- Processed control material
- Control DNA

■価 格

在庫：2009年09月30日 11:15 現在

メーカー	商品コード	品名	包装	価格(¥)	在庫	保存条件	詳細情報
		説明文				薬規制等	
		発売カタログ					
BTL	91.111	BIOFOOD Standard Kit (24 tests)	1kit	45,000	お問合せ		
□		この製品の取扱は終了いたしました。					
BTL	91.112	BIOFOOD Standard Kit (48 tests)	1kit	58,000	お問合せ	-20℃	
□		この製品の取扱は終了いたしました。					
BTL	91.121	BIOFOOD Identification Kit, Standard Control (Chicken, Goat, Pig, Turkey) (24 tests)	1kit	49,000	お問合せ	-20℃	
□		この製品の取扱は終了いたしました。					
BTL	91.122	BIOFOOD Identification Kit, Standard Control (Chicken, Goat, Pig, Turkey) (48 tests)	1kit	62,000	お問合せ	-20℃	
□		この製品の取扱は終了いたしました。					
		ニュース 2007年10月15日号(No.600) (p. 5)					
BTL	91.131	BIOFOOD Mixed Kit (24 tests)	1kit	49,000	お問合せ		
□		この製品の取扱は終了いたしました。					
BTL	91.132	BIOFOOD Mixed Kit (48 tests)	1kit	62,000	お問合せ	-20℃	
□		この製品の取扱は終了いたしました。					

>> 注文書内容を見る

ページトップへ

■Genomic DNA Extraction Kit

在庫：2009年09月30日 11:15 現在

メーカー	商品コード	品名	包装	価格(¥)	在庫	保存条件	詳細情報
		説明文				薬規制等	

BTL	91.011	Control DNA, Horse, BIOFOOD Identification	500ng	14,000	お問合せ	4℃	
□		この製品の取扱は終了いたしました。					
		総合カタログ2005-2006[試薬編]製品リスト (p. 249) 遺伝子工学 研究用試薬・機器 2004 (p. 461) ニュース 2003年11月 1日号 (No.303) (p. 7)					
BTL	91.014	Control DNA, Human, BIOFOOD Identification	500ng	14,000	お問合せ	4℃	
□		この製品の取扱は終了いたしました。					
		総合カタログ2005-2006[試薬編]製品リスト (p. 249) 遺伝子工学 研究用試薬・機器 2004 (p. 461) ニュース 2003年11月 1日号 (No.303) (p. 7)					
BTL	91.004	Control DNA, Pig, BIOFOOD Identification	500ng	14,000	お問合せ	-20℃	
□		この製品の取扱は終了いたしました。					
		総合カタログ2005-2006[試薬編]製品リスト (p. 249) 遺伝子工学 研究用試薬・機器 2004 (p. 461) ニュース 2003年11月 1日号 (No.303) (p. 7)					
BTL	91.010	Control DNA, Rabbit, BIOFOOD Identification	500ng	14,000	お問合せ	4℃	
□		この製品の取扱は終了いたしました。					
		総合カタログ2005-2006[試薬編]製品リスト (p. 249) 遺伝子工学 研究用試薬・機器 2004 (p. 461) ニュース 2003年11月 1日号 (No.303) (p. 7)					
BTL	91.005	Control DNA, Sheep, BIOFOOD Identification	500ng	14,000	お問合せ	-20℃	
□		この製品の取扱は終了いたしました。					
		総合カタログ2005-2006[試薬編]製品リスト (p. 249) 遺伝子工学 研究用試薬・機器 2004 (p. 461) ニュース 2003年11月 1日号 (No.303) (p. 7)					
BTL	91.007	Control DNA, Turkey, BIOFOOD Identification	500ng	14,000	お問合せ	-20℃	
□		この製品の取扱は終了いたしました。					
		総合カタログ2005-2006[試薬編]製品リスト (p. 249) 遺伝子工学 研究用試薬・機器 2004 (p. 461) ニュース 2003年11月 1日号 (No.303) (p. 7)					

>> 注文内容を見る

ページトップへ

■ご購入時のご注意

BIOFOOD Identification Kitは、キットに添付するコントロール DNA を 4 種ご指定いただくことができます。コントロールの指定をご希望の場合は、**当日注文**でのご注文となります。注文書に必要事項をご記入の上、販売店にお渡し下さい。なお、ご指定がない場合は、4 種(chicken, goat, pig, turkey)を組み合わせた標準セットになります。詳細は、下記までお問い合わせ下さい。

[記載日情報:2007/07/04 現在]

◎お問い合わせ先
テクニカルサポート(筑波担当)
[TEL] 03-5684-1820 [FAX] 03-5684-1775

Partial Translation of Appendix 1
(http://www.funakoshi.co/jp/node/10898)

Translation of the section" BIOFOOD Identification Kit"

--BIOFOOD Identification Kit

This kit is to identify animal species for animal-derived components in a sample. First, the primer pair against the sequence common to vertebrates is used to perform PCR, and then the amplified DNA is digested with three restriction enzymes and subjected to gel electrophoresis. Animal species for animal-derived components in the sample are identified based on the electrophoresis patterns of RFLP (Restriction Fragment Length Polymorphism).

Sensitivity: 0.5% to %% or more

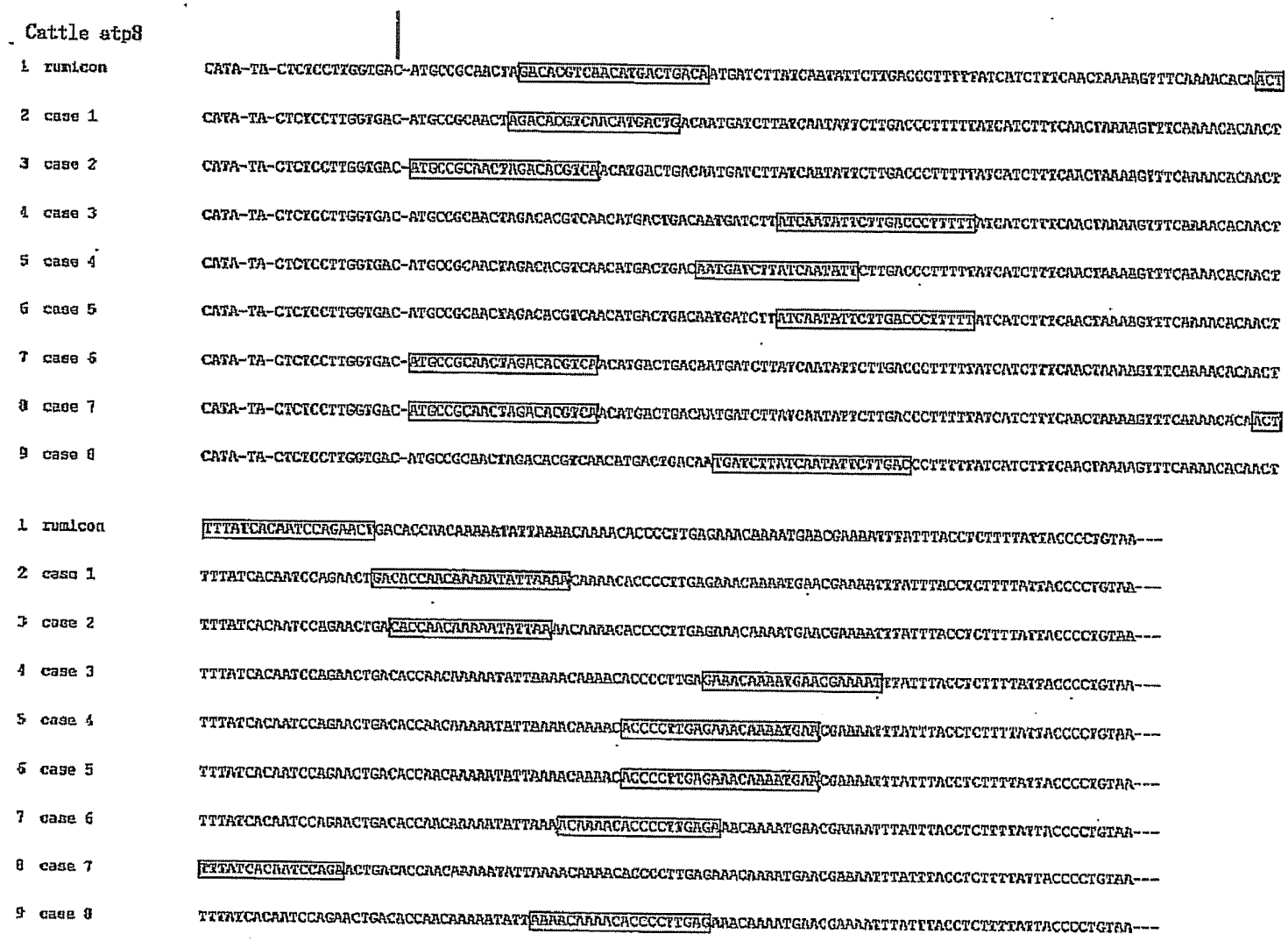
*For a sample containing components derived from three or more of animal species, it may be difficult to give an electrophoresis pattern.

Translation of the legend to photograph of RFLP pattern (Fig. 1 above)

Example of RFLP analysis

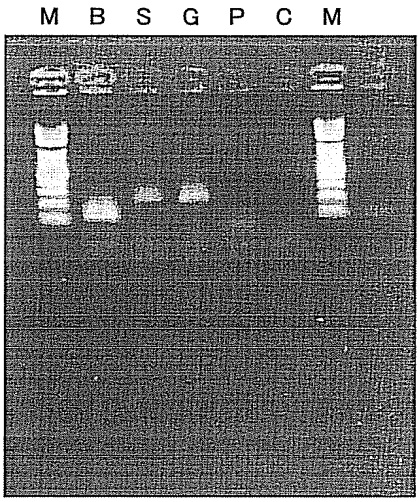
DNA was purified from various samples, amplified by PCR, and and then digested with Enzyme 1 of BIOFOOD Identification Kit and subjected to agarose gel electrophoresis. By combining this result with the other results obtained by using the other restriction enzymes included in the Kit, animal species for animal-derived components in the sample can be identified.

Lane 1: buffalo; Lane 2: rabbit; Lane 3: horse; Lane 4: roe deer; Lane 5: cattle; Lane 6: Goat; Lane 7: deer; Lane 8: emu; Lane 9: red kangaroo; Lane 10: ostrich; Lane 11: cat; lane 12: dog; lane 13: muse; lane 14: human; lane M: 100 bp size marker.

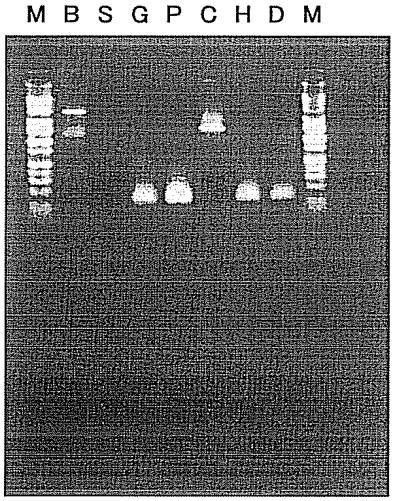

Cattle atp8
1 rumlcon
2 case 1
3 case 2
4 case 3
5 case 4
6 case 5
7 case 6
8 case 7
9 case 8

Appendix 3

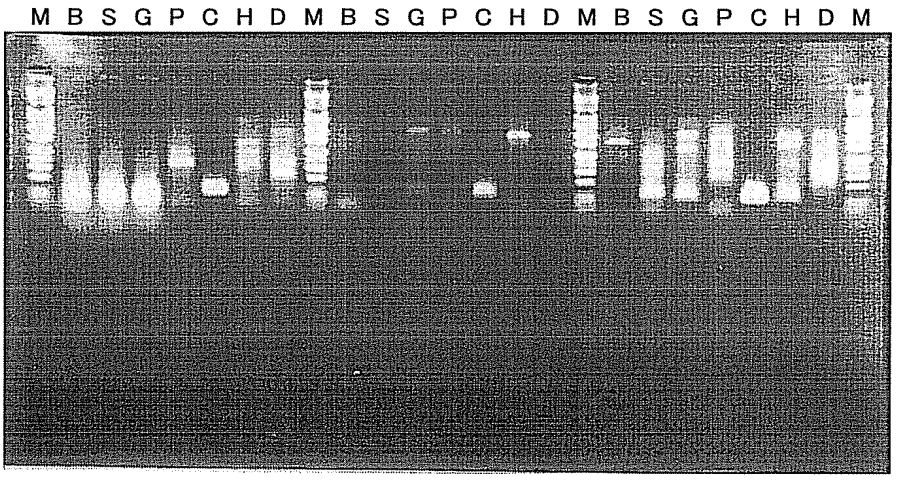
Case 1



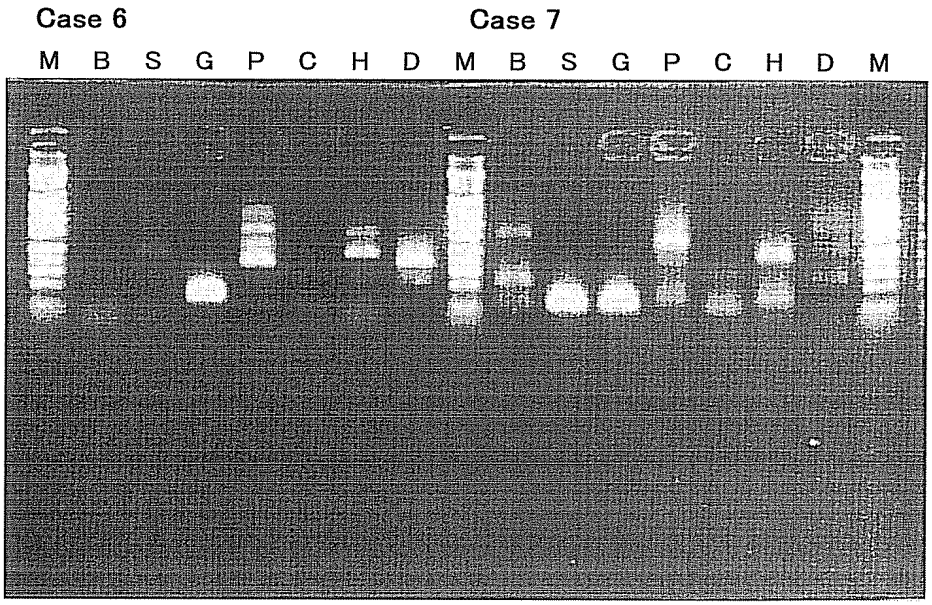
Case 2



Case 3 Case 4 Case 5

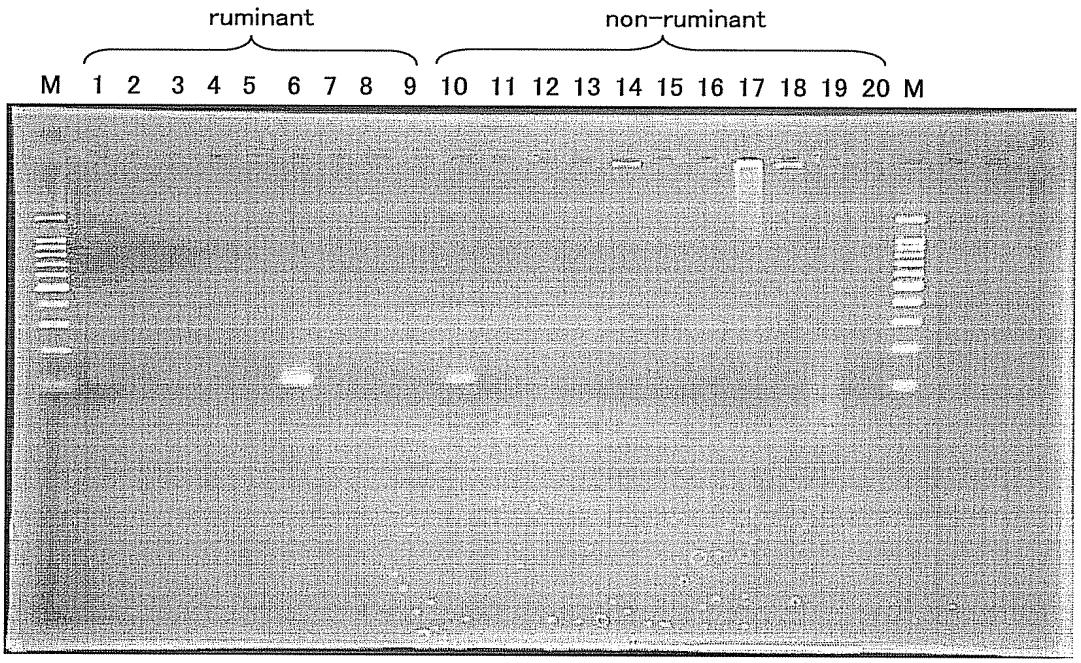


M: 100 bp DNA size markers
B: Cattle, S: Sheep, G: Goat, P: Pig, C: Chicken, H: Horse, D: Deer



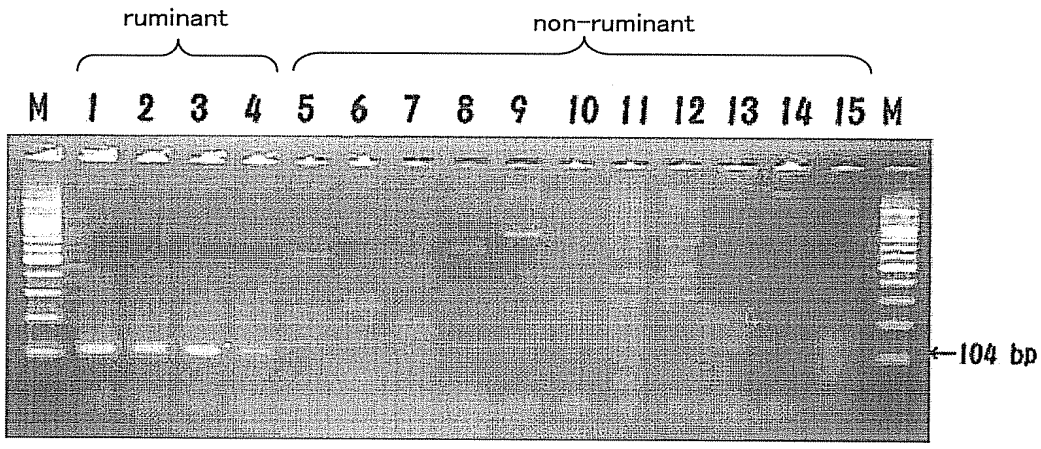
M: 100 bp DNA size markers
B: Cattle, S: Sheep, G: Goat, P: Pig, C: Chicken, H: Horse, D: Deer

Case 8



1–5: Cattle, 6: Sheep, 7: Goat, 8–9: Deer,
10: Swine, 11: Horse, 12: Rabbit, 13: Whale, 14: Poultry, 15: Codfish,
16: Salmon, 17: Sardine, 18: Crab, 19: Prawn, 20: Shelfish,
M: 100 bp DNA size markers

rumicon (claimed primer pair): same as Fig. 7 in the specification



1: Cattle, 2: Sheep, 3: Goat, 4: Deer,
5: Swine, 6: Horse, 7: Rabbit, 8: Whale, 9: Poultry, 10: Codfish,
11: Salmon, 12: Sardine, 13: Crab, 14: Prawn, 15: Shelfish,
M: 100 bp DNA size markers